

## Enzyme addition to animal feed for the reduction of environmental nitrogenous discharge

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### Abstract

Animal feed is supplemented with an enzyme, especially beta-glucanase, xylanase or phytase, in order to reduce nitrogenous discharge, especially ammonia, to the atmosphere.

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CLAIMS

1. Use in an animal feed of an enzyme for reducing gaseous nitrogenous discharges to the atmosphere.
2. Use according to claim 1 wherein discharge of ammonia to atmosphere is reduced.
3. Use according to claim 1 or 2 where the feed comprises barley.
4. Use according to any one of claims 1 to 3 wherein the said enzyme is  $\beta$ -glucanase.
5. Use according to any one of claims 1 to 4 wherein the said feed is chicken feed.
6. Use according to claim 1 substantially as hereinbefore described.

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(54) Enzyme addition to animal feed for the reduction of environmental nitrogenous discharge

(57) Animal feed is supplemented with an enzyme, especially beta-glucanase, xylanase or phytase, in order to reduce nitrogenous discharge, especially ammonia, to the atmosphere.

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REDUCING NITROGENOUS DISCHARGES

The present invention relates to animal feed compositions, and more particularly to animal feed compositions which give rise to reduced nitrogenous discharges and thus protect the air and the environment.

It is known, for example according to the article by Hesselman, K. and Åman P. which appeared in Animal Feed Science and Technology, 15 (1986) 83-93, that  $\beta$ -glucanase has a favourable effect on the feeding of poultry, mainly on their growth and especially on the conversion index, especially when their feed consists of barley. This article discloses no effect on the environment when enzymes are added to animal feeds.

The present invention provides a new use of enzymes, in addition to the effect on the growth of animals, when enzymes are added to animal feeds. It has been found that the presence of enzymes makes it possible to reduce the amount of nitrogenous discharges and, more particularly to reduce gaseous nitrogenous discharges to the air. As nitrogenous derivatives are harmful to the environment, especially water and/or the air, the reduction of these discharges makes a highly advantageous contribution to protecting the environment.

It has been found that the addition of enzymes makes possible better digestion of the feed and thus reduces the amount of nitrogenous matter contained

in the faeces of animals. These faeces contain a proportion of nitrogenous matter reduced by approximately 7% by weight, with respect to the faeces of animals receiving the same diet without addition of 5 enzymes. It was also found that discharges of ammonia to the atmosphere, which are very large when animal husbandry is carried out under battery conditions, were reduced by at least 50%.

10 The enzymes which can be used in this invention are preferably chosen from for example, xylanases,  $\beta$ -glucanases or phytases.

15 These enzymes have a marked effect on the environment when the feed consists mostly of cereals such as barley, oats, wheat or rya. The enzymes can be supplied to the animal in various forms; they can thus be used in the pulverulent, granulated or liquid form.

20 It is apparent that, when the enzyme is supplied in granulated form, it is necessary to verify the amount of enzyme available to the animal, because granulation has a tendency to destroy part of the enzymes. When the enzyme is supplied in liquid or pulverulent form, it is necessary carefully to verify the homogeneity of distribution of the enzyme in the feed.

25 The enzyme is supplied in a proportion by weight which can vary according to the feed used. When, for example, the feed consists mainly of barley, an amount of  $\beta$ -glucanase of between 100 and 200 units per

kilo of feed can be supplied, which represents approximately one gram of enzyme per kilo of feed.

The supplemented feed is then distributed to the animals being reared such as broiler fowl, laying hens, turkeys, piglets or pigs.

5 The invention is illustrated by the following Examples .

EXAMPLE

The experiment is divided into two 10 experimental periods (called P1 and P2) with three diets per period (0, 80 and 100 units of  $\beta$ -glucanase). The same test is carried out 16 times for each diet with 45 chickens per test. For each period, the animals are fed from day D0 to day D7 with a standard growth feed; from day D7, they are divided into groups and receive the experimental feeds, up to day D28. 15 distributed in the form of meal. The period P2 is carried out using the same litter as P1, the animals which had been fed for 28 days being replaced by a second batch of animals which had been fed for 8 days. 20 Measurements were carried out after 19, 21, 26 and 28 days.

The feeds used the following compositions:

COMPOSITION (kg/100 kg)		A	B	C
5	Barley	50.0	50.0	50.0
	Maize	9.7	9.7	9.7
	Soya bean cake	27.5	27.5	27.5
	Meat meal	4.0	4.0	4.0
	Corn oil	5.0	5.0	5.0
	Calcium carbonate	0.5	0.5	0.5
	Calcium hydrogenphosphate	1.3	1.3	1.3
	Premix	1.0	1.0	1.0
10	Premixture	1.0	1.0	1.0
	$\beta$ -Glucanase (units/kg)	0	80	100

The measurement of the ammonia content of the air, measured using Draeger tubes (see publication by Conceicão which appeared in British Poultry Science, 1989 30: 765-776), in the 0-10 ppm range, using a constant volume of air in a hermetically sealed enclosure, showed that the air in the regions where the animals received diets containing 80 or 100 units of  $\beta$ -glucanase contained a lower proportion of ammonia.

20 Influence of  $\beta$ -glucanase supplementation on the ammonia content of the air during period 1 (after 28 days) and period 2 (after 19 days)

	DIET	PERIOD P1		PERIOD P2	
		NH <sub>3</sub> CONTENT in mg/m <sup>3</sup>	RELATIVE NH <sub>3</sub> CONTENT	NH <sub>3</sub> CONTENT in mg/m <sup>3</sup>	RELATIVE NH <sub>3</sub> CONTENT
25	DIET A 0 unit of $\beta$ -glucanase	1.18	100%	5.75	100%
	DIET B 80 units of $\beta$ -glucanase	0.77	65.3%	3.95	68.7%
30	DIET C 100 units of $\beta$ -glucanase	0.55	47.2%	2.57	44.6%

Although the method used for measuring the

ammonia content of the air (Draeger tubes) contains a 15% of risk error, the quality of the air is nevertheless significantly improved ( $p<0.001$ ) in the regions where the animals receiving a diet supplemented 5 with  $\beta$ -glucanase are situated.

Influence of the  $\beta$ -glucanase supplementation on the ammonia content of the air during Period 2

	DIET	1st series (D19)		2nd series (D21)		3rd series (D26)		4th series (D28)	
		ppm	%	ppm	%	ppm	%	ppm	%
10	A 0 units of $\beta$ -glucanase	3.30a	100	7.18a	100	34.45a	100	111.62a	100
	B 80 units of $\beta$ -glucanase	5.57b	68.7	4.96ab	69.1	17.56ab	51.3	23.79b	21.3
	C 100 units of $\beta$ -glucanase	3.62b	44.6	3.45b	48.1	7.10b	29.6	22.26b	19.9
15	Statistical significance	**		*		**		**	

In each column, the different values modified by a letter are statistically different at the 5% level.  
\*, \*\*, and \*\*\* indicate significant effects at the 5%, 2% and 0.1% levels respectively.

$\beta$ -Glucanase thus reduces the ammonia content in the air, especially at a supplementation level of 100 units/kg of feed. This effect becomes more marked as the condition of the litter becomes more soiled:  
25 about -64% (i.e.  $100-\frac{1}{2} (51.3 + 21.3)$ ) and about -75% (i.e.  $100-\frac{1}{2} (29.6 + 19.9)$ ) for diets containing 80 and 100 units/kg of feed respectively during the last week of P2.

CLAIMS

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5. Use according to claim 1 or 2 where the feed comprises barley.
3. Use according to any one of claims 1 to 3 wherein the said enzyme is  $\beta$ -glucanase.
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**Patents Act 1977**  
**Examiner's report to the Comptroller under Section 17**  
**(The Search report)**

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**Relevant Technical Fields**

- (i) UK Cl (Ed.N) C3H (HK1, HK4)  
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Search Examiner  
MR C SHERRINGTON

Date of completion of Search

Databases (see below)  
 (i) UK Patent Office collections of GB, EP, WO and US patent specifications.  
 (ii) ONLINE: WPI, CLAIMS, DIALOG/BIOTECH

Documents considered relevant following a search in respect of Claims :-  
 1 to 6

**Categories of documents**

- |    |   |    |   |
|----|---|----|---|
| X: | Document indicating lack of novelty or of inventive step.   | P: | Document published on or after the declared priority date but before the filing date of the present application.        |
| Y: | Document indicating lack of inventive step if combined with one or more other documents of the same category. | E: | Patent document published on or after, but with priority date earlier than, the filing date of the present application. |
| A: | Document indicating technological background and/or state of the art.   | S: | Member of the same patent family; corresponding document.   |

Category	Identity of document and relevant passages		Relevant to claim(s)
X	EP 0468596 A1	(GIST-BROCADES N.V.) whole document	1, 2, 6
P, X	EP 0619369 A1	(AVEVE N.V.) whole document	1, 2, 6
X	WO 91/04673 A1	(NOVO NORDISK A/S ET AL) whole document	1, 2, 5, 6
X	US 5314692	(CULTOR LTD) whole document	1 to 6
X	US 3880742	(GLAXO LABORATORIES LIMITED) whole document, especially column 1, lines 12 to 21, column 5, line 62 to column 6, line 17; example 6; Claim 13	1 to 6
X	Anim. Feed Sci. Technol. 1986, 15, 83-93 - The effect of beta-glucanase on the utilisation of starch and nitrogen by...		1 to 6
X	Feed Management 1992, 43(1), 8-17 Phytase: The value of improving phosphorus retention		1 to 3, 5, 6
X	Livestock Production Science 1992, 31, 75-94 Alteration of nutrition as a means to reduce environmental pollution by pigs		1 to 4, 6
X	Agro Food Industry Hi-Tech 1993, 4(1), 27-29 Using enzymes to improve the productive value of poultry and pig feedstuffs		1 to 6

Databases: The UK Patent Office database comprises classified collections of GB, EP, WO and US patent specifications as outlined periodically in the Official Journal (Patents). The on-line databases considered for search are also listed periodically in the Official Journal (Patents).

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**Patents Act 1977**  
**Examiner's report to the Comptroller under Section 17**  
**(The Search report)**

Application number  
GB 9502249.7

Continuation page

Category	Identity of document and relevant passages	Relevant to claim(s)
X	Zootechnica International 1993, 16(2), 42-50 Improving phosphorus retention with phytase	1, 2, 5, 6
X	J. Animal Sci. 1994, 72(1), 126-132 Apparent Digestibility and Retention of Nutrients Bound for Phytate Complexes as Influenced by Microbial Phytase...	1, 2, 6